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Atty. Dkt. No. 038602-1260

THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Axel ULLRICH et al.
Title: NOVEL MEGAKARYOCYTIC PROTEIN TYROSINE KINASES
Appl. No.: 09/977,260
Filing Date: 10/16/2001
Examiner: Unassigned
Art Unit: 1647

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the present Application, Applicant respectfully requests that the above-identified prior application be amended as follows:

In the Specification:

Please amend the specification as follows:

On page 9, delete the 4th, 5th and 6th paragraphs, and replace these paragraphs with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

Figure 9. Shared amino acid sequence homology of MKK1 SEQ ID NO: 2 and csk SEQ ID NO: 7.

Figures 10A and 10B SEQ ID NOS 4, 8-10 respectively, in order of appearance.
Shared amino acid sequence homology of MKK2 and atk/btk.

Figures 11A, 11B, 11C and 11D SEQ ID NOS 6, 11-19, respectively, in order of appearance.

On page 13, delete the 3rd and 4th paragraphs, and replace these paragraphs with following in accordance with 37 CFR § 1.121. A marked up version showing changes made is attached:

The nucleotide and deduced amino acid sequence of human MKK1, MKK2, and MKK3 are shown in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and 3A-3B (SEQ ID NOS 5-6), respectively. Figures 9 (SEQ ID NOS 2 and 7, respectively, in order of appearance), 10A-10B (SEQ ID NOS 4, 8-10, respectively, in order of appearance) and 11A-11D (SEQ ID NOS 6, 11-19, respectively, in order of appearance) show the shared sequence homology between MKKs and related tyrosine kinases.

5.1 The MKK Coding Sequences

The nucleotide coding sequence and deduced amino acid sequence of the human MKK1, MKK2, and MKK3 genes are depicted in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and 3A-3B (SEQ ID NOS 5-6), respectively. In accordance with the invention, any nucleotide sequence which encodes the amino acid sequence of an MKK gene product can be used to generate recombinant molecules which direct the expression of an MKK.

On pages 13-14, delete the last paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

In a specific embodiment described herein, the human MKK1, MKK2, and MKK3 genes were isolated by performing polymerase chain reactions (PCR) in combination with two degenerate oligonucleotide primer pools that were designed on the basis of highly conserved sequences within the kinase domain of receptor tyrosine kinases corresponding to the amino acid sequence HRDLAA (residues 350-355 of SEQ ID NO: 2) (sense primer) and [SDVWS/FY] SDVWSF/Y (SEQ ID NO:24) (antisense primer) (Hanks *et al.*, 1988). The MKK cDNAs were synthesized by reverse transcription of poly-A RNA from the human K-562 cell line, ATCC accession number CCL 243, or from the Meg 01 cell line, (Ogura *et al.*, Blood 66: 1384 (1985)).

Atty. Dkt. No. 038602-1260

On page 14, delete 1st paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

The PCR fragments were used to screen a lambda gt11 library of human fetal brain. For each individual MKK, several overlapping clones were identified. The composite of the cDNA clones for MKK1, MKK2, and MKK3 are depicted in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) , and 3A-3B (SEQ ID NOS 5-6), respectively.

On pages 37-38, delete the last paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes made is attached:

cDNA was used in a polymerase chain reaction under standard conditions (*PCR Technology-Principles and Applications for DNA Amplifications*, H.E. Erlich, Ed., Stockton Press, New York 1989). Degenerate pools of primers corresponding to the amino acid sequence HRDLAA (residues 350-355 of SEQ ID NO:2) and SDVWSF/Y (SEQ ID NO:24) were prepared and used for the amplification:

5' oligo pool

	H	R	D	L	A	A	
5' GGAATTCC	CAC	AGN	GAC	TTN	GCN	GCN	AG 3' (<u>SEQ ID NO: 20</u>)
	T C	A	T C	A	A	C	

3' oligo pool

	F/Y	S	W	V	D	S	
5' GGAATTCC	GAA	NGT	CCA	NAC	GTC	NGA	3' (<u>SEQ ID NO: 21</u>)
	ATG	CA		C	C		

Thirty-five PCR cycles were carried out using 8 µg (0.8 µg) of the pooled primers. (Annealing 55°C, 1 min; Extension 72°C, 2 min; Denaturation 94°C, 1 min). The reaction product was subjected to polyacrylamide gelelectrophoresis. Fragments of the expected size (~210 bp) were isolated, digested with the restriction enzyme EcoRI, and subcloned into the pBluskript vector (Stratagene) using standard techniques (*Current*

Protocols in Molecular Biology, eds. F.M. Ausubel *et al.*, John Wiley & Sons, New York, 1988).

On page 38, delete the last paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

The partial cDNA sequence of the new MKK1 TK, which was identified by PCR, was used to screen a λ gt11 library from human fetal brain cDNA (Clontech)(complexity of 1×10^{10} recombinant phages). One million independent phage clones were plated and transferred to nitrocellulose filters following standard procedures (Sambrook, H.J., Molecular Cloning, Cold Spring Harbor Laboratory Press, USA, 1989). The filters were hybridized to the EcoRI/EcoRI fragment of clone MKK1, which had been radioactively labeled using 50 μ Ci [α^{32} P]ATP and the random-primed DNA labeling kit (Boehringer Mannheim). The longest cDNA insert of ~3500 bp was digested with the restriction enzymes EcoRI/SacI to obtain a 5' end probe of 250 bp. This probe was used to rescreen the human fetal brain library and several overlapping clones were isolated. The composite of the cDNA clones of MKK1, MKK2 and MKK3 is shown in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and 3A-3B (SEQ ID NOS 5-6), respectively. The 1.75 million independent phage clones of a human placenta library, λ ZAP, were plated and screened with the 5' end probe (EcoRI/SacI) of the clone used above. Subcloning of positive bacteriophages clones into pBluskript vector was done by the *in vivo* excision protocol (Stratagene).

On page 39, delete the 2nd paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

The composite cDNA sequence and the predicted amino acid sequence of MKK1, MKK2 and MKK3 are shown in Figures 1A-1B (SEQ ID NOS 1-2), 2A-2B (SEQ ID NOS 3-4) and 3A-3B (SEQ ID NOS 5-6), respectively.

On page 41, delete the 1st paragraph, and replace this paragraph with the following in accordance with 37 CFR § 1.121. A marked up version showing changes is attached:

8. Example: Production of Anti-MKK Antibodies and Immunoprecipitation of MKK Antibodies and Immunoprecipitation of MKK

Antibodies recognizing MKK1 and MKK2 protein were made in rabbits using standard procedures. The anti-carboxy terminus MKK1 antibody was generated using the synthetic peptide GQDADGSTSPRSQEP (SEQ ID NO 22). The amino-terminus MKK1 Ab was generated using a GST-fusion proteins containing 78 amino acids coded by the Smal to BG12 fragment of the MKK1 gene. The anti-carboxy terminus MKK2 Ab was made using a synthetic peptide corresponding to the sequence QQLSSIEPLREKDKH (SEQ ID NO 23).

In the Claims:

In accordance with 37 CFR § 1.121, please substitute for original claims 15, 17, and 19 the following rewritten versions of the same claims, as amended. The changes are shown explicit in the attached "Version with Markings to Show Changes Made."

15. (Amended) The isolated recombinant MKK1 of Claim 14 comprising the amino acid sequence depicted in Figures 1A and 1B (SEQ ID NOS 1-2).

17. (Amended) The isolated recombinant MKK2 of Claim 16 comprising the amino acid sequence depicted in Figures 2A and 2B (SEQ ID NOS 3-4).

19. (Amended) The isolated recombinant MKK3 of Claim 18 comprising the amino acid sequence depicted in Figures 3A and 3B (SEQ ID NOS 5-6).

REMARKS

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application.

Applicant believes that the present application is now in condition for allowance. Favorable consideration of the application as amended is respectfully requested.

Atty. Dkt. No. 038602-1260

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Date: 5/8/02

FOLEY & LARDNER
Customer Number: 22428



22428

PATENT TRADEMARK OFFICE

Telephone: (202) 672-5475

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Respectfully submitted,

By: 

Beth A. Burrous
Attorney for Applicant
Registration No. 35,087

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Marked up replacement paragraphs:

Page 9, delete the 4th, 5th and 6th paragraphs:

Figure 9. Shared amino acid sequence homology of MKK1 SEQ ID NO: 2 and csk SEQ ID NO: 7.

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Pages 13-14, delete the last paragraph:

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with two degenerate oligonucleotide primer pools that were designed on the basis of highly conserved sequences within the kinase domain of receptor tyrosine kinases corresponding to the amino acid sequence HRDLAA (residues 350-355 of SEQ ID NO: 2) (sense primer) and [SDVWS/FY] SDVWSF/Y (SEQ ID NO:24) (antisense primer) (Hanks *et al.*, 1988). The MKK cDNAs were synthesized by reverse transcription of poly-A RNA from the human K-562 cell line, ATCC accession number CCL 243, or from the Meg 01 cell line, (Ogura *et al.*, Blood 66: 1384 (1985)).

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5' oligo pool

	H	R	D	L	A	A	
5'	G	G	A	A	T	T	C
	C	A	C	A	G	T	T
	T	C	A	T	C	A	A
							C

3' (SEQ ID NO: 20)

3' oligo pool

	F/Y	S	W	V	D	S	
5'	G	G	A	A	T	T	C
	G	A	A	T	T	C	
	A	T	G	C	A	N	A
							C

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Thirty-five PCR cycles were carried out using 8 µg (0.8 µg) of the pooled primers. (Annealing 55°C, 1 min; Extension 72°C, 2 min; Denaturation 94°C, 1 min). The reaction product was subjected to polyacrylamide gelelectrophoresis. Fragments of the

expected size (~210 bp) were isolated, digested with the restriction enzyme EcoRI, and subcloned into the pBluscript vector (Stratagene) using standard techniques (*Current Protocols in Molecular Biology*, eds. F.M. Ausubel *et al.*, John Wiley & Sons, New York, 1988).

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Atty. Dkt. No. 038602-1260

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Marked up rewritten claims:

15. (Amended) The isolated recombinant MKK1 of Claim 14 comprising the amino acid sequence depicted in Figures 1A and 1B (SEQ ID NOS 1-2).

17. (Amended) The isolated recombinant MKK2 of Claim 16 comprising the amino acid sequence depicted in Figures 2A and 2B (SEQ ID NOS 3-4).

19. (Amended) The isolated recombinant MKK3 of Claim 18 comprising the amino acid sequence depicted in Figures 3A and 3B (SEQ ID NOS 5-6).